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Preparation and application of a new modified liquid chromatographic chiral stationary phase based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid

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Abstract

As an effort to improve the chiral recognition efficiency of a previously reported chiral stationary phase (CSP) based on (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid, a new CSP was prepared by simply replacing the amide N–H hydrogens of the tethering groups of the old CSP with methyl groups. The new CSP was superior to the old one in the resolution of racemic primary amines. However, in the resolution of α-amino acids and amino alcohols, the new and the old CSPs were complementary with each other. The elution orders on the new CSP were sometimes opposite to those on the old one. Consequently, the chiral recognition mechanism on the new CSP was presumed to be different from that on the old one. The chiral recognition behavior of the new CSP were investigated with four selected analytes and found to be dependent to some extent on the content of organic and acidic modifiers in aqueous mobile phase and the column temperature.

Keywords: Chiral stationary phase, LC; Enantiomer separation; (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid

1. Introduction

Two types of chiral crown ethers immobilized on solid support have been most widely utilized as chiral stationary phases (CSPs) for the liquid chromatographic resolution of racemic compounds containing a primary amino group.

The first type is the one related to 1,1′-binaphthyl-based chiral crown ethers. For example, optically active bis-(1,1′-binaphthyl)-22-crown-6 covalently bonded on silica gel or optically active (3,3′-diphenyl-1,1′-binaphthyl)-20-crown-6 dynamically coated on octadecyl silica gel have been developed as CSPs for the resolution racemic compounds containing a primary amino group by Cram [1,2] and Shinbo [3,4]. One of them has been commercialized as the CROWNPAK CR (Daicel Chemical Industries) for the chromatographic resolution of racemic compounds containing a primary amino group [5–8]. Recently, optically active (3,3′-diphenyl-1,1-binaphtyl)-20-crown-6 covalently bonded to silica gel was also developed as a new CSP in our laboratory and successfully applied to the resolution of various racemic α-amino acids, amines, amino alcohols and related primary amino compounds [9,10].

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Another type of chiral crown ether successfully used as a chiral selector is (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, **1**, (Fig. 1a), which was fist developed by Lehn and co-workers [11]. For example, Machida and co-workers developed a CSP by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, **1**, to silica gel [12]. While the CSP developed by Machida and co-workers shows certain structural ambiguity, we prepared a structurally well defined CSP **2** (Fig. 1b) by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, **1**, to 3-aminopropylsilica gel via a simple two step procedure [13,14]. CSP **2** was excellent in resolving α-amino acids and their derivatives [14], racemic amines and amino alcohols [15], racemic fluoroquinolone antibacterials [13,16,17] and even racemic secondary amines including β-blockers [18].

As an effort to improve the chiral recognition efficiency of CSP **2**, we noted the possibility of the intramolecular hydrogen bonds between N–H hydrogens of the two connecting amide tethers of the CSP and the ether oxygens of the crown ether ring as shown in Fig. 1c from previous studies [19,20]. The two hydrogen bonds shown in Fig. 1c are expected to hinder the complex formation between the crown ether ring and the ammonium ion ($R–NH_3^+$). Consequently, removal of the two hydrogen bonds shown in Fig. 1c might improve the chiral recognition ability of the CSP. Based on this rationale, in this study, we prepared CSP **3** (Fig. 1d) and applied it to

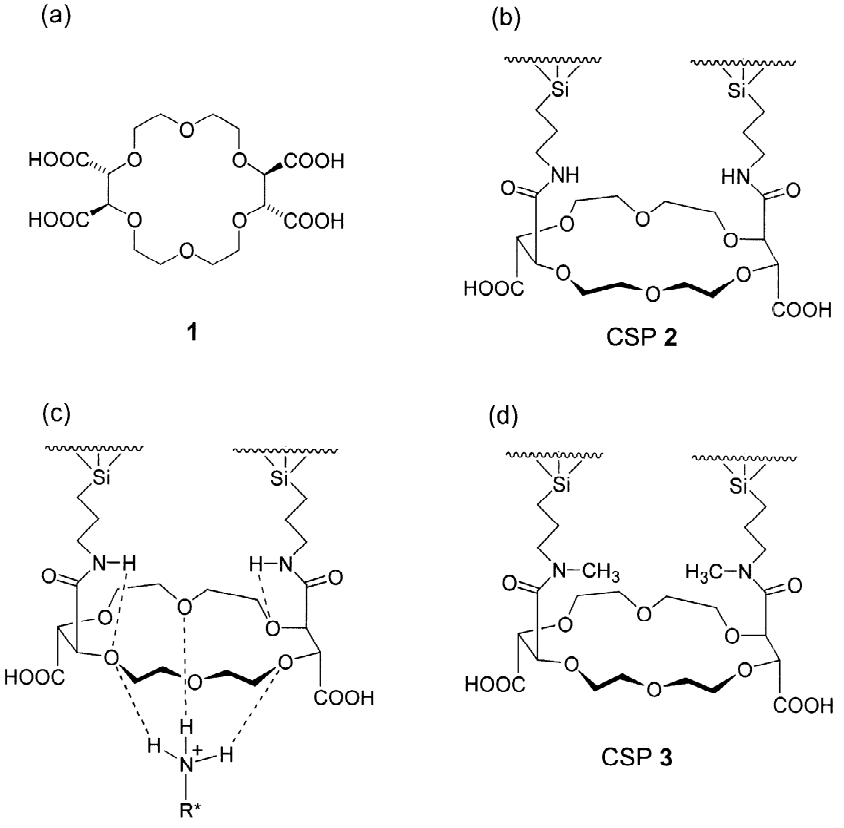


Fig. 1. (a) Structure of (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid **1**. (b) Structure of CSP **2**. (c) Two intramolecular hydrogen bonds between the amide N–H hydrogen of the two connecting tethers of CSP **2** and the ether oxygens of the crown ether moiety of the CSP. These two intramolecular hydrogen bonds are expected to hinder the complex formation of the protonated analytes ($R–NH_3^+$) with the crown ether ring. (d) Structure of CSP **3**.

the resolution of various α-amino acids, amines and amino alcohols containing a primary amino group.

2. Experimental

2.1. Preparation of CSP 3 and column packing

CSP **3** was prepared by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** to 3-(*N*-methylamino)propyl silica gel via the same procedure as that for the preparation of CSP **2** [14] using 2,6-lutidine instead of triethylamine. 3-(*N*-Methylamino)propylsilica gel used in this study was prepared as following. A 250-ml two-neck flask equipped with a Dean-Stark trap, a condenser and a magnetic stirring bar was charged with silica gel (7 g, Kromasil 5 μm, 100 Å, 340 m^2/g) and toluene (150 ml). The heterogeneous mixture was heated to reflux until the azeotropic removal of water was complete and then 3-(*N*-methylamino)propyltrimethoxysilane (7 ml, Lankaster, 95%) was added. The whole mixture was heated to reflux for 72 h. The modified silica gel was filtered, washed successively with methanol, ethyl acetate, methylene chloride, hexane and diethyl ether and then dried under high vacuum.

Based on the elemental analysis of 3-(*N*-methylamino)propylsilica gel and CSP **3**, the surface concentration of the chiral selector calculated according to the equation reported previously [21] was 0.60 $\mu mol/m^2$.

CSP **3** thus prepared was slurried in methanol and packed into a 150×4.6 mm stainless steel HPLC column by using a conventional slurry packing method with an Alltech slurry packer.

2.2. Chromatography

Chromatography was performed with an HPLC system consisting of a Waters model 515 HPLC pump, a Rheodyne model 7725i injector with a 20-μl sample loop, a Waters 2487 UV Dual λ Absorbance Detector and a YoungLin Autochro Data Module (Software: YoungLin Autochro-WIN 2.0 plus). The temperature of the chiral column was controlled by using a JEIO TECH VTRC-620 Circulator (Seoul, Korea).

Analytes used in this study were available from previous studies [14,15] or purchased from Aldrich. Injection samples were prepared by dissolving analytes in methanol at a concentration of 1.0 mg/ml and an injection size of 3 μl was typically used. Elution orders were determined by injecting configurationally known samples available from Aldrich.

3. Results and discussion

CSP **3** was applied in resolving various α-amino acids **4**, amines **5** and amino alcohols **6**, all of which contain a primary amino group. The chromatographic resolution results are summarized in Tables 1–3 and the typical chromatograms are presented in Fig. 2. For the purpose of comparison, the resolution data on CSP **2** are included in Tables 1–3. Most data on CSP **2** were quoted from previous studies [14,15] and some other data, which were not reported previously, were newly obtained in this study. All resolution results on CSP **3** summarized in Tables 1–3 were obtained under an identical resolution condition by using a mixed solvent of methanol–water (50:50, v/v) containing sulfuric acid (10 m*M*) at 20 °C. The elution orders shown in Tables 1–3 were determined by injecting configurationally known samples.

Comparison of the resolution results on CSP **3**, which are summarized in Tables 1–3, with those on CSP **2** shows somewhat interesting results. Even though all α-amino acids tested were resolved reasonably well on CSP **3** except for proline, which does not contain a primary amino group, CSP **3** is generally worse than CSP **2** in the resolution of α-amino acids **4** as shown in Table 1. However, in the resolution of arginine, aspartic acid, cysteine, histidine, isoleucine, serine and threonine, CSP **3** is better than CSP **2**. Especially, aspartic acid, cysteine and isoleucine are resolved quite well with reasonable separation factors on CSP **3** while these are resolved with only marginal separation factors on CSP **2**. To the contrary, in the resolution of racemic amines **5**, CSP **3** is always superior to CSP **2** in terms of the separation (α) and resolution (R_S) factors as shown in Table 2. In the resolution of amino alcohols **6**, CSP **3** is not always better than CSP **2** as shown in Table 3. In the resolution of some

Table 1
Comparison of the resolution of α-amino acids on CSP **2** and CSP **3**[a]

	Amino acids	CSP **2**			CSP **3**		
		k_1	α	R_S	k_1	α	R_S
4a	Alanine	1.37 (L)	1.28	1.33	2.24 (L)	1.23	1.13
4b	Arginine	1.46 (L)	1.48	1.91	3.24 (L)	1.43	2.58
4c	Asparagine	1.31 (L)	1.10	0.63	1.42 (L)	1.06	0.39
4d	Aspartic acid	1.51 (L)	1.22	1.25	2.16 (L)	1.38	2.57
4e	Cysteine	1.32 (L)	1.10	0.30	2.46 (L)	1.21	1.62
4f	Glutamic acid	1.30 (L)	1.44	1.78	3.41 (L)	1.20	1.40
4g	Glutamine	1.31 (L)	1.32	1.72	2.31 (L)	1.25	1.59
4h	Histidine	2.28 (L)	1.48	1.33	3.39 (L)	1.40	3.03
4i	Isoleucine	0.47 (L)	1.19	0.75	0.88 (D)	1.55	3.06
4j	Leucine	0.73 (L)	1.32	1.42	2.15 (L)	1.10	0.62
4k	Methionine	1.27 (L)	1.39	2.06	3.15 (L)	1.12	0.75
4l	Phenylalanine	0.88 (L)	1.48	2.31	2.23 (L)	1.19	1.53
4m	Phenylglycine	2.07 (L)	2.25	6.46	8.95 (L)	1.49	4.39
4n	Proline	0.12	1.00		0.14	1.00	
4o	Serine	1.94 (L)	1.52	1.63	1.86 (D)	2.58	6.99
4p	Threonine	0.24 (D)	1.42	1.30	0.41 (D)	1.78	3.43
4q	Tryptophan	0.92 (L)	1.44	2.15	2.83 (L)	1.16	1.37
4r	Tyrosine	0.84 (L)	1.44	2.00	2.45 (L)	1.16	1.45
4s	Valine	0.40 (L)	1.31	1.14	1.99 (L)	1.21	1.14
4t	2-Aminobutanoic acid	0.72	1.34	2.06	1.51	1.23	1.54
4u	2-Aminopetanoic acid (norvaline)	0.73	1.36	1.89	1.78	1.13	0.92
4v	2-Aminohenenoic acid (norleucine)	0.70	1.34	1.67	1.99	1.11	0.80

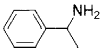
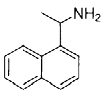
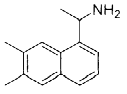
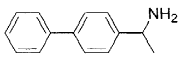
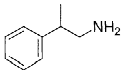
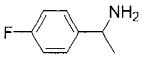
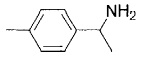
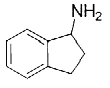
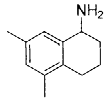
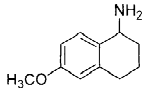
[a] All data on CSP **2** were quoted from Ref. [14]. Chromatographic conditions on CSP **3** are as following. Mobile phase, 50% methanol in water + sulfuric acid (10 m*M*). Flow-rate, 0.5 ml/min. Detection, 210 nm UV. Temperature, 20 °C. k_1, Retention factor of the first eluted enantiomer. In the parenthesis, the absolute configuration of the first eluted enantiomer is presented. α, Separation factor. R_S, Resolution factor.

amino alcohols (**6c**, **6d**, **6e**, **6g**), CSP **3** is better than CSP **2**. However, in the resolution of other amino alcohols (**6a**, **6b**, **6f**), CSP **2** is better than CSP **3**. Especially, amino alcohol **6a** is not resolved at all on CSP **3**, but resolved with reasonable separation (α) and resolution factor (R_S) on CSP **2**. Amino alcohols **6c**, **6d** and **6e** are not resolved at all on CSP **2**, but resolved on CSP **3**. From the results shown in Table 1–3, it is concluded that CSP **2** and CSP **3** are complementary with each other in the resolution of α-amino acids **4** and amino alcohols **6**. However, in the resolution of racemic primary amines **5**, CSP **3** is always superior to CSP **2** and consequently, CSP **3** is much more useful than CSP **2** and can be solely used without complementary use of CSP **2**.

Another interesting results to note on the two CSPs are the elution orders. The elution orders for the resolution of isoleucine, serine and threonine on CSP **3** are reversed compared to those for the resolution of other amino acids while the elution orders only for the resolution of threonine on CSP **2** are reversed as shown in Table 1. In the resolution of α-phenylethylamine (**5a**), β-phenylpropylamine (**5e**) and α-(4-methylphenyl)ethylamine (**5g**) on CSP **3,** the elution orders are reversed compared to those on CSP **2** as shown in Table 2. In addition, the elution orders for the resolution of amino alcohols **6b** and **6f** on CSP **3** are reversed compared to those on CSP **2** as shown in Table 3. From these results concerning the elution orders, the chiral recognition mechanism on CSP **3** might be presumed to be different from that on CSP **2**. However, which factor is responsible for the different chiral recognition mechanism on the two CSPs is not clear yet.

In order to see the effect of mobile phase modifiers on the resolution behavior of CSP **3**, we selected four analytes, which were resolved well on CSP **3**, including serine (**4o**), α-(4-methylphenyl)ethylamine (**5g**), 1-aminoindan (**5h**) and phenylglycinol (**6b**) and resolved them on CSP **3** by

Table 2
Comparison of the resolution of racemic amines on CSP **2** and CSP **3**[a]

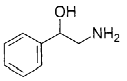
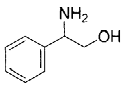
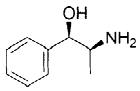
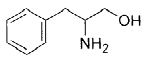
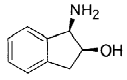
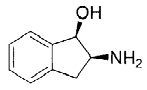
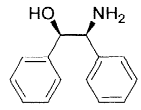
	Amines	CSP **2**			CSP **3**		
		k_1	α	R_S	k_1	α	R_S
5a		2.45 (R)	1.10	0.80	6.39 (S)	1.49	3.91
5b		1.90 (S)	1.28	2.57	8.62 (S)	1.84	5.65
5c		1.38	1.84	5.23	7.05	2.56	8.53
5d		2.86	1.11	1.05	9.11	1.34	2.46
5e		1.40 (S)	1.11	1.02	6.95 (R)	1.12	1.05
5f		0.42	1.22	0.82	7.88	1.26	2.31
5g		0.41 (R)	1.11	0.38	5.93 (S)	1.41	3.55
5h		1.16 (R)	1.55	3.27	2.32 (R)	2.92	10.53
5i		0.51	1.38	1.09	0.95	2.53	8.00
5j		0.49	1.67	1.92	0.99	3.08	8.76
5k		0.51	1.39	1.69	0.94	2.94	8.06

[a] All data on CSP **2** were quoted from Ref. [15] except for the underlined data. Underlined data were newly obtained. Chromatographic conditions are as following. Mobile phase, 50% methanol in water + sulfuric acid (10 m*M*). Flow-rate, 0.5 ml/min. Detection, 210 nm UV. Temperature, 20 °C. k_1, Retention factor of the first eluted enantiomer. In the parenthesis, the absolute configuration of the first eluted enantiomer is presented. α, Separation factor. R_S, Resolution factor.

varying the type and content of organic and acidic modifiers in aqueous mobile phase. The chromatographic results are summarized in Table 4.

As shown in Table 4, the retention factors (k_1) depend very much on the content of the organic and acidic modifier in aqueous mobile phase. As the content of organic modifier (methanol) in aqueous mobile phase increases, the retention factors (k_1) increase quite a lot (see entries a–c in Table 4). These trends are exactly consistent with those of

Table 3
Comparison of the resolution of racemic amino alcohols on CSP **2** and CSP **3**[a]

	Amino alcohols	CSP **2**			CSP **3**		
		k_1	α	R_S	k_1	α	R_S
6a		1.10	1.40	1.52	9.47	1.00	
6b		1.44 (S)	1.35	2.18	3.84 (R)	1.31	2.93
6c		0.21	1.00		2.36 (1R,2S)	1.19	1.96
6d		0.18	1.00		1.69 (S)	1.42	3.46
6e		0.11	1.00		0.90 (1S,2R)	1.09	0.62
6f		1.98 (1R,2S)	1.78	0.80	2.94 (1S,2R)	1.12	1.32
6g		0.29 (1S,2R)	1.53	1.48	4.47 (1S,2R)	2.29	7.67

[a] All data on CSP **2** were quoted from Ref. [15] except for the underlined data. Underlined data were newly obtained. Chromatographic conditions are as following. Mobile phase, 50% methanol in water + sulfuric acid (10 m*M*). Flow-rate, 0.5 ml/min. Detection, 210 nm UV. Temperature, 20 °C. k_1, Retention factor of the first eluted enantiomer. In the parenthesis, the absolute configuration of the first eluted enantiomer is presented. α, Separation factor. R_S, Resolution factor.

CSP **2** reported previously [14,15,22]. As the content of organic modifier increases, the aqueous mobile phase becomes less polar and more hydrophobic. In this instance, the hydrophilic interaction between polar-protonated analytes and the mobile phase decreases and consequently, the retention is expected to increase as the organic content in the aqueous mobile phase increases. However, the trends of the separation (α) and resolution (R_S) factors with the variation of the content of organic modifier in aqueous mobile phase were not significant. Instead of methanol, acetonitrile was also tried as an organic modifier. Comparison of the two organic modifiers at the constant resolution condition (see entries j and k in Table 4) shows that methanol is more useful than acetonitrile in terms of resolution factor (R_S).

The acidic modifier added to mobile phase has been known to protonate the primary amino group of analytes and facilitate the complexation of the resulting ammonium ion ($R–NH_3^+$) inside the cavity of chiral crown ether moiety [23]. The effect of acidic modifier in mobile phase on the enantioselectivity exerted by CSP **3** was investigated with the variation of the type and content of an acidic modifier in aqueous mobile phase at a constant concentration of organic modifier, methanol (50%) at 20 °C. The chromatographic results are included in Table 4 (see entries b, d–j). First of all, a comparison of the

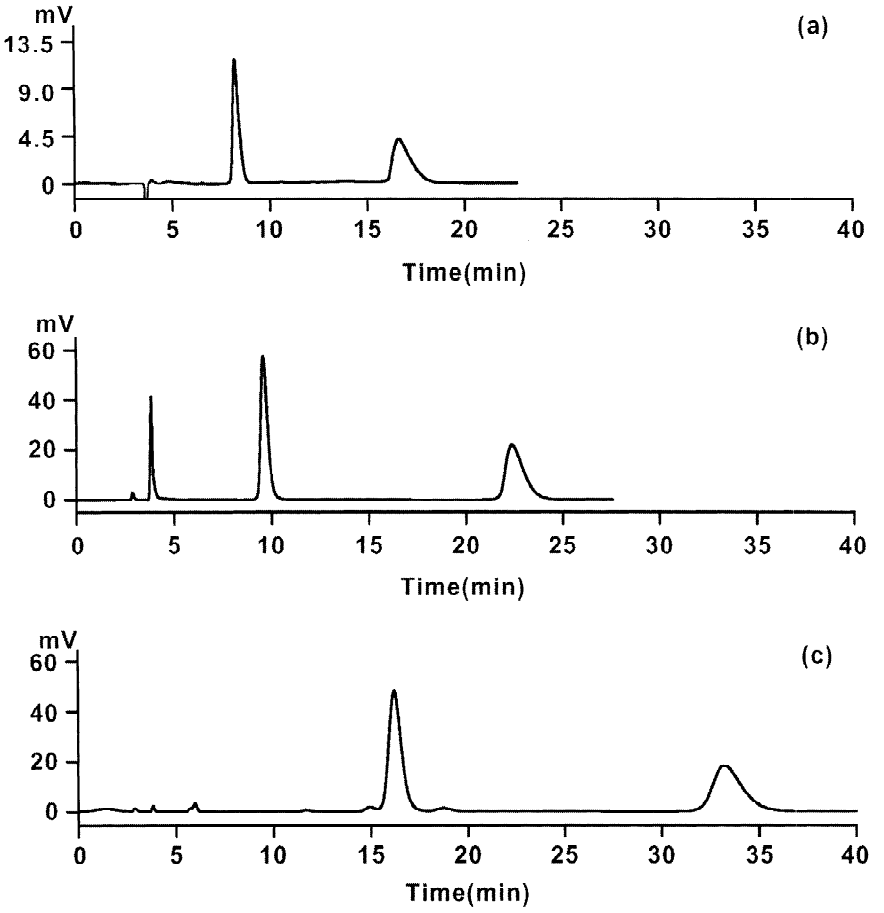


Fig. 2. Typical chromatograms for the resolution of (a) serine **4o**, (b) 1-aminoindan **5h** and (c) 2-amino-1,2-diphenylethanol **6g** on CSP **3**. Mobile phase, 50% methanol in water + sulfuric acid (10 m*M*). Flow-rate, 0.5 ml/min. Detection, 210 nm UV. Temperature, 20 °C.

resolution results obtained with the use of sulfuric acid (entry b), acetic acid (entry d) and perchloric acid (entry j) of identical concentration demonstrates that sulfuric acid and perchloric acid are equally effective as an acidic modifier in terms of both the separation (α) and resolution (R_S) factors while acetic acid is relatively less effective. The most surprising results are the reversed elution orders for the resolution of phenylglycinol (**6b**) with the use of acetic acid as an acidic modifier. As shown in Table 4, the (*R*)-enantiomer of phenylglycinol (**6b**) is always eluted first when sulfuric acid or perchloric acid is used as an acidic modifier. However, when acetic acid is used as an acidic modifier, the (*S*)-enantiomer is eluted first. The chiral recognition mechanism seems to be variable with the type of acidic modifier, but the rationalization for the reversed elution orders might need further study.

The effect of the content of acidic modifier in aqueous mobile phase on the chromatographic resolution behavior of CSP **3** can be seen from the resolution data obtained with the variation of the content of acidic modifier in aqueous mobile phase. As shown in Table 4, as the content of sulfuric acid increases, the retention factors (k_1) for the resolution of the four-selected analytes on CSP **3** decrease continuously while the separation (α) and resolution (R_S) factors do not show significant trends (see entries b and e–g). As the content of acidic modifier in aqueous mobile phase increases, the ionic strength

Table 4
Resolution of selected racemic compounds (**4o**, **5g**, **5h** and **6b**) on CSP **3** with the variation of the type and content of organic and acidic modifier in aqueous mobile phase[a]

	Mobile phase	**4o**			**5g**			**5h**			**6b**		
		k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
a	30% CH_3OH + H_2SO_4 (10 m*M*)	0.71 (*R*)	2.76	6.67	2.98 (*S*)	1.46	3.68	1.31 (*R*)	2.74	8.05	1.87 (*R*)	1.37	2.87
b	50% CH_3OH + H_2SO_4 (10 m*M*)	1.86 (*R*)	2.58	6.99	5.93 (*S*)	1.41	3.55	2.32 (*R*)	2.92	10.53	3.84 (*R*)	1.31	2.93
c	80% CH_3OH + H_2SO_4 (10 m*M*)	3.97 (*R*)	2.72	4.53	14.59 (*S*)	1.34	3.63	4.81 (*R*)	2.88	11.18	8.75 (*R*)	1.20	1.71
d	50% CH_3OH + AcOH (10 m*M*)	1.67 (*R*)	2.02	4.11	1.07 (*S*)	1.14	0.41	0.47 (*R*)	1.89	2.15	0.71 (*S*)	1.35	0.96
e	50% CH_3OH + H_2SO_4 (1 m*M*)	3.01 (*R*)	3.11	6.87	10.37 (*S*)	1.46	3.95	4.06 (*R*)	2.81	9.20	7.52 (*R*)	1.25	2.32
f	50% CH_3OH + H_2SO_4 (5 m*M*)	2.00 (*R*)	2.88	6.20	7.61 (*S*)	1.44	1.75	3.05 (*R*)	2.90	9.64	5.18 (*R*)	1.32	2.94
g	50% CH_3OH + H_2SO_4 (20 m*M*)	1.30 (*R*)	2.59	5.64	5.14 (*S*)	1.36	3.33	2.06 (*R*)	2.84	10.20	3.22 (*R*)	1.32	3.25
h	50% CH_3OH + $HClO_4$ (1 m*M*)	2.25 (*R*)	2.78	4.04	0.84 (*S*)	1.26	0.88	0.34 (*R*)	2.32	3.05	0.77	1.00	
i	50% CH_3OH + $HClO_4$ (5 m*M*)	1.39 (*R*)	2.92	5.11	2.66 (*S*)	1.46	2.67	1.06 (*R*)	2.82	6.56	1.78 (*R*)	1.22	1.46
j	50% CH_3OH + $HClO_4$ (10 m*M*)	1.30 (*R*)	2.83	5.34	3.64 (*S*)	1.48	3.28	1.47 (*R*)	2.92	8.97	2.65 (*R*)	1.29	1.86
k	50% CH_3CN + $HClO_4$ (10 m*M*)	0.60 (*R*)	2.52	3.92	0.92 (*S*)	1.49	2.87	0.39 (*R*)	2.94	6.03	0.68 (*R*)	1.25	1.58

[a] Flow-rate, 0.5 ml/min. Detection, 210 nm UV. Temperature, 20 °C. k_1, Retention factor of the first eluted enantiomer. In the parenthesis, the absolute configuration of the first eluted enantiomer is presented. α, Separation factor; R_S, Resolution factor.

of mobile phase increases and consequently, the hydration or the dissolution of polar-protonated analytes by mobile phase is expected to increase. In this instance, polar-protonated analytes are eluted faster and faster as the content of acidic modifier increases.

However, as the content of perchloric acid in aqueous mobile phase increases, the retention factors (k_1) decrease continuously only for the resolution of serine (**4o**) on CSP **3** while the retention factors (k_1) increase for the resolution of α-(4-methylphenyl)ethylamine (**5g**), 1-aminoindan (**5h**) and phenylglycinol (**6b**) (see entries h–j in Table 4). As the content of perchloric acid in aqueous mobile phase increases, the separation (α) and resolution (R_S) factors do not show significant trends for the resolution of serine (**4o**), but show increasing trends for the resolution of α-(4-methylphenyl)ethylamine (**5g**), 1-aminoindan (**5h**) and phenylglycinol (**6b**). The retention behavior of relatively more polar-protonated analyte, serine (**4o**), on CSP**3** with the use of perchloric acid as an acidic modifier is the same as that with the use of sulfuric acid as an acidic modifier. However, the retention trends of relatively less polar-protonated analytes, α-(4-methylphenyl)ethylamine (**5g**), 1-aminoindan (**5h**) and phenylglycinol (**6b**), on CSP **3** with the use of perchloric acid as an acidic modifier are opposite to those with the use of sulfuric acid as an acidic modifier. The perchlorate anion has been known to be quite lipophilic [24]. In this instance, the lipophilic interactions of the ion pair formed between less polar-protonated analytes and perchlorate anion with the CSP are expected to be an important factor for the retention of analytes and consequently, the retention factors (k_1) increase continuously as the content of perchloric acid in aqueous mobile phase increases.

The effect of the column temperature on the enantioselectivities for the selected four analytes on CSP **3** is summarized in Table 5. As shown in Table 5, the retention (k_1) and separation (α) factors increase as the column temperature decreases. However, the trends of the resolution factors (R_S) with the variation of the column temperature are not significant. At lower temperature, the formation of the diastereomeric complexes between the individual enantiomers of an analyte and the chiral crown ether moiety of the CSP is expected to become more favorable and this is significant with the more stable diastereomeric complex. Consequently, the retention (k_1) and separation (α) factors increase as the column temperature decrease.

In summary, in this study, we prepared a new crown ether-based CSP (CSP **3**) by simply replacing the amide N–H hydrogens of the tethering group of the previously reported CSP (CSP **2**) with methyl groups. CSP **3** was superior to CSP **2** in the resolution of primary amines. However, CSP **2** and CSP **3** were complementary with each other in the

Table 5
Resolution of selected racemic compounds (**4o**, **5g**, **5h** and **6b**) on CSP **3** with the variation of the column temperature[a]

Temperature (°C)	**4o**			**5g**			**5h**			**6b**		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
20	1.86 (*R*)	2.58	6.99	5.93 (*S*)	1.41	3.55	2.32 (*R*)	2.92	10.53	3.84 (*R*)	1.31	2.93
10	2.50 (*R*)	3.04	6.96	10.54 (*S*)	1.44	3.36	4.02 (*R*)	3.12	10.23	6.91 (*R*)	1.34	2.90
5	3.05 (*R*)	3.22	7.22	13.38 (*S*)	1.46	3.21	4.91 (*R*)	3.24	9.09	8.76 (*R*)	1.36	2.81

[a] Mobile phase, 50% methanol in water + sulfuric acid (10 m*M*). Flow-rate, 0.5 ml/min. Detection, 210 nm UV. k_1, Retention factor of the first eluted enantiomer. In the parenthesis, the absolute configuration of the first eluted enantiomer is presented. α, Separation factor; R_S, Resolution factor.

resolution of α-amino acids and amino alcohols. Even though the chiral crown moiety of the two CSPs has the same absolute configuration, the elution orders on CSP **3** were different from those on CSP **2** in some cases, indicating that the chiral recognition mechanisms on the two CSPs might be not identical. In order to see the effect of the organic and acidic modifiers in aqueous mobile phase and the column temperature on the enantioselectivities exerted by CSP **3**, we selected four racemic analytes and resolved them on CSP **3** with the variation of the type and content of the organic and acidic modifiers in aqueous mobile phase and the column temperature. The chromatographic resolution results for the four selected analytes demonstrated that chromatographic parameters such as retention (k_1), separation (α) and resolution (R_S) factors could be controllable to some extent.

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